BD™ Middlebrook 7H10 Agar

INTENDED USE
BD Middlebrook 7H10 Agar is used for the isolation and cultivation of mycobacteria.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE
Microbiological method.
During this century, many culture media have been devised for the cultivation of mycobacteria. The early ones were egg-based formulations and included Löwenstein-Jensen Medium and Petragnani Medium. Dubos and Middlebrook were instrumental in the development of a number of formulations which contained oleic acid and albumin as key ingredients to aid in the growth of the tubercle bacilli and to protect the organisms against a variety of toxic agents.[1] Subsequently, Middlebrook and Cohn improved the formulation of oleic acid-albumin agar and obtained faster, more luxuriant growth of *Mycobacterium* species on their medium designated as 7H10.[2,3] It has been reported that the 7H10 medium tends to grow fewer contaminants than the egg-based media commonly used for the cultivation of mycobacteria.[4]
In **BD Middlebrook 7H10 Agar** a variety of inorganic salts provides substances essential for the growth of mycobacteria. The sodium citrate, when converted to citric acid, serves to hold certain inorganic cations in solution. Glycerol is an abundant source of carbon and energy. Oleic acid, as well as other long chain fatty acids, can be utilized by tubercle bacilli and plays an important role in the metabolism of mycobacteria. Catalase destroys toxic peroxides that may be present in the medium. The primary effect of albumin is that of protection of the tubercle bacilli against toxic agents and, therefore, it enhances their recovery on primary isolation. Partial inhibition of contaminating bacteria is achieved by the presence of the malachite green dye.

REAGENTS
BD Middlebrook 7H10 Agar
Formula* Per Liter Purified Water

Magnesium Sulfate	0.025 g	Bovine Albumin V	5.0 g
Ferric Ammonium Citrate	0.04	Catalase	0.004
Sodium Citrate	0.4	Pyridoxine	0.001
Ammonium Sulfate	0.5	Zinc Sulfate	0.001
Monosodium Glutamate	0.5	Copper Sulfate	0.001
Disodium Phosphate	1.5	Biotin	0.0005
Monopotassium Phosphate	1.5	Calcium Chloride	0.0005
Agar	17.0	Malachite Green	0.00025
Sodium Chloride	0.85	Glycerol	5.0
Glucose	2.0	Oleic Acid	0.05 ml

pH 6.6 +/- 0.2
*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS
IVD . For professional use only.
Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.
Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.
Laboratory procedures involving mycobacteria require special equipment and techniques to minimize biohazards.[5-7] Biosafety Level 3 is required for handling of specimens and cultures.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.
Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate at 35 +/- 2° C aerobically or in an aerobic atmosphere supplemented with carbon dioxide.

Strain	Incubation	Result
Mycobacterium tuberculosis H37Ra ATCC™ 25177	2 to 3 weeks	Growth good to excellent
Mycobacterium fortuitum DSM 46621	2 to 3 weeks	Growth good to excellent
Mycobacterium smegmatis DSM 43061	2 to 5 days	Growth good to excellent
Mycobacterium terrae ATCC 15755	2 to 3 weeks	Growth fair to excellent
Staphylococcus aureus ATCC 25923	2 to 5 days	Inhibited
Escherichia coli ATCC 25922	2 to 5 days	Inhibited
Uninoculated	Amber, very slight greenish hue	

PROCEDURE

Materials Provided

BD Middlebrook 7H10 Agar (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This medium is used for the isolation and cultivation of mycobacteria from clinical specimens and may also be used for the cultivation of mycobacteria in special tests (e.g., for testing of disinfectants against mycobacteria).
For details on suitable specimens, consult the references.[6-10]

Test Procedure

The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria. N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle but effective digesting and decontaminating agent. For detailed instructions on decontamination and cultivation methods consult the references.[4-10]
Following inoculation, keep plates shielded from light and place plates, medium side down, in a **BD GasPak™** jar operated with a **GasPak™** disposable carbon dioxide generator envelope, or other suitable system providing an aerobic atmosphere enriched with carbon dioxide. Incubate at 35 ±2°C. Avoid desiccation of the medium during incubation.
Note: Cultures from skin and soft tissue lesions suspected to contain *M. marinum*, *M. ulcerans*, *M. haemophilum, M. chelonae,* or other species with a lower temperature optimum must be incubated at 25 to 33°C. Incubate a duplicate culture at 35 to 37°C.[5,9]

Results

Plates may be read within 5 to 7 days after inoculation and once a week thereafter for up to 8 weeks. For details on colony morphology and pigmentation, consult the references.[6-10]
For reading plates, invert the plates on the stage of a dissecting microscope. Read at 10-60x magnification with transmitted light. Scan rapidly at 10-20x for the presence of colonies. Higher magnification (30-60x) is helpful in observing colony morphology, i.e., serpentine cord-like colonies.
Record the following observations: [5,6]

1. Number of days required for colonies to become macroscopically visible.
2. Number of colonies:
   No colonies = Negative
   Less than 50 colonies = Record actual count
   50-100 colonies = 1+
   100-200 colonies = 2+
   Almost confluent (200-500) = 3+
   Confluent (more than 500) = 4+
3. Pigment production
   White, cream or buff = Nonchromogenic (NC)
   Lemon, yellow, orange, red = Chromogenic (Ch)

Stained smears will show acid-fast bacilli, which are reported only as "acid-fast bacilli" unless definitive tests are performed. Consult the appropriate references for information on further differential tests and on procedures for complete identification of the organisms isolated.[4-10]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Middlebrook 7H10 Agar is one of the standard agar media for the isolation and cultivation of mycobacteria from clinical specimens.[4,6,7-11]
Since this medium is only partially selective, bacteria other than mycobacteria may grow if specimens are not appropriately pretreated for decontamination.[6,7,9,10]

Negative cultures do not rule out active infection by mycobacteria. For good practices, several different media formulations (solid and liquid) should be inoculated.[6-10]

REFERENCES

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PACKAGING/AVAILABILITY

BD Middlebrook 7H10 Agar

Cat. No. 254520	Ready-to-use Plated Media, cpu 20
Cat. No. 254521	Ready-to-use Plated Media, cpu 120

FURTHER INFORMATION

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